ANAlyzing animal model and drug-loaded microspheres for local breast cancer recurrence in autologous fat grafting

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INTRODUCTION
Autologous fat grafting has been recently reevaluated in breast augmentation and breast reconstruction after surgery (1, 2). Fat grafting is an innovative method to use in breast reconstruction for cancer patients; it is less invasive, more natural-looking, and can cause fewer immunological problems. It has less complications such as ruptures, malposition, and capsular contractures, compared with silicone breast implants.

In addition to the preferred fat grafting for reconstructive purposes, there is interest in using adipose-derived stem cells for the regenerative process. In 2001, adipose-derived stem cells (ASCs) were found as mesenchymal stem cells in fat tissue. Because of their abilities of multi-lineage differentiation and their wound healing effects, ASC-enriched fat grafting was developed. It is reported that the ASC-enriched fat graft retention is better than retention without ASCs (3, 4).

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There is however major concern that this fat grafting with adipose-derived stem cells may cause cancer cell progression. Microspheres loaded with chemotherapeutic drugs have been an on-going study as a solution to the concern of the stem cells’ effects on cancer cell progression. Microspheres are small spherical particles with diameters in the micron range, hence the name. They have a history of being used for drug delivery by slowing secreting the encapsulated contents since they can also be less invasive and help evenly spread treatment over a specific area. Using these polymer-based, drug-loaded microspheres has proved to enhance drug delivery compared to other methods (5). Anti-cancer drugs (Doxorubicin and Paclitaxel) were encapsulated into microspheres and tested with multiple assays to determine effects on cell progression.

OBJECTIVE
The aim of this study was to develop an animal model for breast cancer recurrence in fat grafting and to analyze our doses of cancer cells in fat tissue to quantify what effect the cells’ environment will have on cell progression with a computer software. In addition to this analysis, the objective was to also determine the efficiency of chemo-therapeutic microspheres to be used as a future solution to possible cancer cell progression.

HYPOTHESIS/SUCCESS CRITERIA
The hypothesis of the study is that the NIS Elements software will help effectively quantify the effect of adipose stem cells on cell proliferation of cancer cells. This will result on a further fat grafting study with chemotherapeutic drug-loaded microspheres, which will show significant cancer cell death when added to cancer cell samples.

METHOD
For the fat grafting study, an exact dose of MDA-MB-231 or BT-474 breast cancer cells were mixed with human fat tissue and injected into the subcutaneous of 8 to 10 week old, immune-deficient female mice, injecting the same doses with Matrigel as a positive control. After 6 or 8 weeks, the mice were euthanized and samples were taken to be weighed, and analyzed histologically with H&E, human-specific pan-cytokeratin, and Ki67. Analysis was focused on the Ki67 staining because of its focus on cell proliferation; it is a protein that marks the proliferation and is therefore helpful for identifying growth of the cancer cell populations in each injection. NIS Elements software was then used to determine the percentage of proliferating cancer cells in each sample from the mice. Pictures were obtained from the four samples of fat tissue on each mice to be analyzed with the software. The software can differentiate the pigmentation of the stained slides of samples from the mice injections. In Ki67 staining, proliferated areas appear brown while the rest of the tissue appears a blue/purple. The brown areas were highlighted and compared to the total overall imaged area to obtain a percentage of proliferation.

For the microsphere portion of the study, 125 milliliters of an aqueous solution containing a chemotherapeutic drug was encapsulated in either a poly (dl-lactide-co-glycolide) solution for single-walled microspheres or in a poly(l-lactide) solution then dispersed in poly (dl-lactide-co-glycolide) for double-walled microspheres. Empty microspheres with no drug encapsulated were used as a control. Those solutions were spun in polyvinyl alcohol, centrifuged to isolate the microspheres, and freeze-dried to eliminate any remaining water. A Cyquant assay and Bioactivity assay were conducted on the microspheres added to cancer cell lines to respectively assess proliferation and biological activity. These assays are based on DNA content, and can give accounts of activity based on cell numbers since the amount of DNA remains constant for any cell line or cell type.
RESULTS
Table 1 below shows a few of the proliferation percentages from the samples. Although the study is still ongoing, results do show differences of proliferation depending on the cells environment. After tumor proliferation percentages were obtained for each image using the NIS software, the data was organized by sample content and dose amount. Since there were a vast quantity of images taken for each sample and dose, the results were averaged (with the standard deviation from the mean shown) for our final data table.

Figure 1 displays a sample of the assay results, specifically the Bioactivity assay, of the empty microspheres in cancer cell samples versus Doxorubicin in cancer cell samples. The assessment of microspheres showed that certain drugs and specific doses work better on certain cancer cells – as seen in the vast difference in final DNA concentration of empty versus drug-loaded microspheres in the MDA-line cancer cells as compared to the MCF line. However, the microspheres do effectively reduce these cancer cells and therefore help prevent cell progression.

Figure 1: By comparing the DNA concentration (mg/mL) to the amount of Doxorubicin (mg) in the microspheres (either empty or loaded); we see that this method of drug delivery can reduce the number of cancer cells seen with the MCF-7 cancer cell line up top, but more significantly in the MDA cell line on the bottom.

<table>
<thead>
<tr>
<th></th>
<th>MDA+Matrigel+Lipo</th>
<th>BT+Media</th>
<th>BT+Matrigel</th>
<th>MDA+Media</th>
<th>MDA+Matrigel</th>
</tr>
</thead>
<tbody>
<tr>
<td>100k dose</td>
<td>11.46±10.08</td>
<td>0</td>
<td>10.95±11.77</td>
<td>19.82</td>
<td>17.26±11.94</td>
</tr>
<tr>
<td>10k dose</td>
<td>5.12±7.80</td>
<td>2.00±4.48</td>
<td>4.59±8.60</td>
<td>0</td>
<td>7.14±11.67</td>
</tr>
</tbody>
</table>

DISCUSSION
The NIS Elements program demonstrates that we can effectively count cancer cells using the software and Ki67 staining technique to help conclude the effects of MDA-MB-231 or BT-474 breast cancer cells and their environment on cancer cell progression. By selecting certain hues of color that have been stained for proliferation, the software provides the ability to use quantitative reasoning for your results as opposed to relying on the human eye. This can be used in further, more specific analysis of the ASC’s effects in fat grafting for cancer patients.

The results of different chemotherapeutic drugs on various cancer cell lines displayed that there seems to be a specific, preferred drug and dose amount in the microspheres that works for each different cell line. Further research may help determine which drug works best with each different type of cancer cell. The assays give us information of the microspheres’ effects on cell progression that may help with determining their role in fat grafting for the future.

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REFERENCES